

T4 DNA Polymerase

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The DNA polymerase encoded by bacteriophage T4 is a well characterized enzyme which is widely used in molecular biology. Here we describe some of the characteristics of the enzyme, its use in generating strand-specific probes, and in generating mutants using both the Kunkel Method and Promega's Altered Sites® II in vitro Mutagenesis System.

Biology of bacteriophage T4

Bacteriophage T4 is a member of the T-even bacteriophages which produce lytic infections in *E. coli*. The study of T-even bacteriophages has played a prominent role in the elucidation of DNA replication mechanisms.

The structure of the bacteriophage T4 is familiar to molecular biologists and is practically an icon of molecular genetics. It consists of an icosahedral head which contains the 166kb genome and a tail segment consisting of tail fibers responsible for viral binding to the *E. coli* cell surface for infection. The viral proteins remain bound to the cell surface while the genome is injected in a syringe-like fashion. This property of bacteriophage T2 was used in early experiments to support the theory of DNA as the genetic material (1).

Once in the cell, the viral genome encodes a number of proteins which serve to disrupt the metabolism of the cell and convert it to the production of virus. Interestingly, bacteriophage T4 is unique among prokaryotes in that some of its messages contain self-excising introns (2-4). The process of infection and gaining control over the host metabolism involves a number of steps (reviewed in reference 5). Briefly, the virus degrades the host chromosome, modifies the host RNA polymerase and reduces the dCTP pool to dCMP. The conversion of dCMP to hydroxymethyl dCMP and its subsequent phosphorylation is essential to the survival of the bacteriophage. This modified cytosine residue is incorporated into the viral genome, making it resistant to host restriction systems. The bacteriophage encodes its own DNA polymerase which, along with several accessory proteins, forms a complex with the ability to replicate the entire genome in 2-5 minutes (reviewed in reference 6).

While the biology of this bacteriophage is interesting, it is the bacteriophage enzymes T4 DNA Polymerase and T4 DNA Ligase which have found particular utility in the field of molecular biology. These enzymes have become standard tools for use in recombinant DNA techniques.

DNA polymerase properties

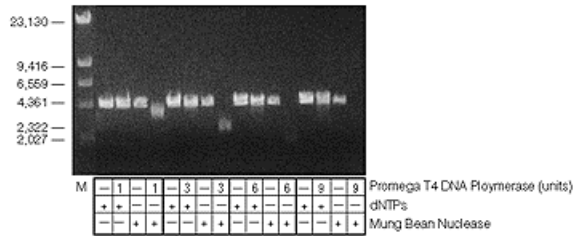
DNA polymerases are widely used for many techniques in molecular biology. Different applications require different enzymatic properties; certain polymerases are best suited for particular techniques. For example, the polymerase chain reaction is best performed with an enzyme that has enhanced thermostability. Polymerases used in these reactions are typically isolated from thermophilic bacteria, such as *Thermus aquaticus*. Nick translations require a 5'→3' exonuclease activity such as that found in DNA Polymerase I from *E. coli*. When this exonuclease domain is removed, the resulting Klenow Fragment is well suited for filling in 3' recessed DNA ends. T4 DNA Polymerase has properties which make it superior for particular applications as well.

T4 DNA Polymerase is a single polypeptide of 103,572Da (7). The enzyme has a template-directed DNA synthesis activity which allows it to extend from the free 3' hydroxyl of a bound primer. Unlike many other polymerases, the T4 DNA Polymerase does not have an intrinsic ability to extend from a nick. This property is important for applications such as site-directed mutagenesis where strand displacement by a polymerase will result in displacement of the mutagenic oligonucleotide. Furthermore, T4 DNA Polymerase lacks any detectable 5'→3' exonuclease activity. This prevents digestion of annealed mutagenic oligonucleotides and reversion to the parental sequence.

T4 DNA Polymerase contains a 3'→5' exonuclease activity which is much stronger than that in Klenow enzyme, and is even more active on single-stranded DNA (ssDNA). [Figure 1](#) demonstrates this activity with a linearized DNA fragment. In the absence of nucleotides, the enzyme rapidly degrades a single strand of the template from each 3' end. This degradation can be seen after treatment with Mung Bean Nuclease to remove the ssDNA comprising the remaining 5' overhang which produces a blunt-ended fragment. The exonuclease activity is responsible for the excellent fidelity of the enzyme, giving a 1,000-fold increase to the intrinsic fidelity of the polymerase activity (8). The overall fidelity of T4 DNA Polymerase is estimated at one misincorporation per 10⁷ residues (9) making it the enzyme of choice for applications where misincorporation is a concern. In contrast, enzymes which lack a 3'→5' exonuclease activity, such as *Taq* DNA Polymerase*, typically have higher misincorporation rates (one per 10⁴ residues).

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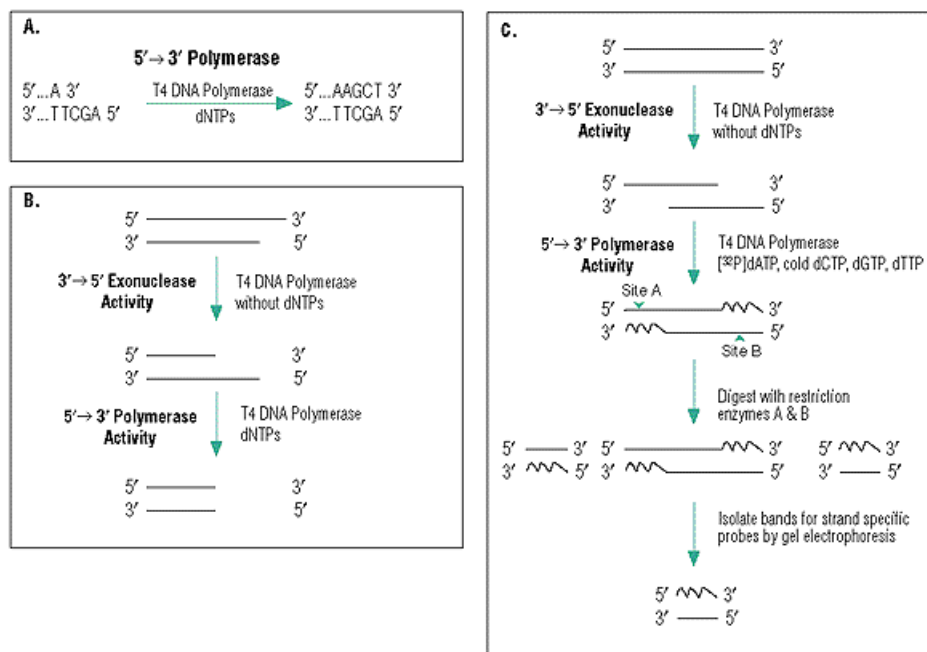
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Figure 1. Examination of the 3'→5' exonuclease activity of T4 DNA Polymerase. Equivalent amounts of *Pst* I linearized pBR322 were incubated for 25 minutes at 37°C, either in the absence of T4 DNA Polymerase or in the presence of the indicated number of units of Promega's T4 DNA Polymerase. The two samples were split into equal aliquots (1.25 μg) and incubated for an additional hour at 37°C, either in the presence or absence of dNTPs (0.4mM of each dNTP, final concentration). Some samples (as indicated in the figure) were then diluted in Mung Bean Nuclease buffer and incubated with 75 units of Mung Bean Nuclease for 10 minutes at 30°C. A total of 0.25 μg of each sample was electrophoresed on a 1% agarose gel.

The 3'→5' exonuclease activity, polymerase activity, template dissociation and misincorporation kinetics of T4 DNA Polymerase have been well characterized (10). The catalytic efficiency of the enzyme is defined both by its intrinsic polymerization rate (estimated at 250nt/second [11]) and its processivity. The processivity of the enzyme appears to vary according to the template tested, with natural templates exhibiting greater processivity than homopolymers (12, 13). On a single-stranded M13 template, with a single primer, a processivity of less than 100 bases per binding event was observed (14). However, when T4 DNA Polymerase was in excess, the apparent processivity was observed to be approximately 800 bases per binding event (11). This increase in apparent processivity may be due to non-specific binding of the polymerase to the DNA template and two dimensional sliding of the polymerase to the elongation site. These studies suggest that enzyme:template ratios are important for achieving complete conversion to double-stranded product. The processivity of the enzyme is greatly enhanced *in vivo* by the addition of accessory proteins such as gp44, gp62, gp45, and the single-stranded binding protein gp32 which act to lock the enzyme onto the DNA template (14).

Applications of T4 DNA Polymerase

T4 DNA Polymerase is widely used in cloning applications and cDNA synthesis to fill-in 3' recessed ends, and to remove protruding 3' ends to produce blunt-ended DNA fragments (Figure 2a and Figure 2b). When incubated with the template in the absence of nucleotides, the 3'→5' exonuclease activity removes protruding 3' ends. After a high concentration (>100 μM) of nucleotides is added, the polymerase fills-in a 3' recessed end.



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Figure 2. Applications utilizing T4 DNA Polymerase. Panel A: Filling of a 3' recessed terminus to produce a blunt end. Panel B: Removal of a protruding 3' terminus in the absence of nucleotides to produce a 3' recessed end, which is then filled-in after

addition of nucleotides. **Panel C:** Creation of strand specific labeled probes by treatment of a linearized fragment with T4 DNA Polymerase in the absence of nucleotides. Recessed ends are filled in the presence of radiolabelled nucleotides and strand-specific probes are isolated after digestion with the appropriate restriction enzymes.

The polymerase can also be used to create strand-specific hybridization probes (Figure 2c). A linear DNA fragment is incubated in the absence of nucleotides to create recessed termini from both ends of the fragment. The recessed ends are made flush in the presence of a labeled nucleotide and the blunt fragment is digested with restriction enzymes to create fragments labeled on one strand.

Most of the available mutagenesis systems use T4 DNA Polymerase to extend the mutant strand from a hybridized mutagenic primer. Since the enzyme has no 5'→3' exonuclease activity or strand displacement activity, the polymerase will not displace the mutagenic primer when the mutant strand synthesis is completed. The high fidelity of the enzyme is also useful in reducing the number of undesirable secondary mutations which can arise in strand synthesis. These secondary mutations are a particular concern when using amplification techniques for incorporating mutations. Figure 3 outlines the use of T4 DNA Polymerase and T4 DNA Ligase in two types of site-directed mutagenesis procedures, Promega's Altered Sites® II *in vitro* Mutagenesis System (16) (Cat.# Q6210) and the Kunkel (15) mutagenesis procedure. In both of these applications, mutagenic oligonucleotides are hybridized to a template strand and extended with T4 DNA Polymerase to create a nicked double-stranded heteroduplex. The nicks are sealed by T4 DNA Ligase and the resulting plasmids are transformed into the appropriate host for mutant selection. Table 1 summarizes data generated using Promega's T4 DNA Polymerase in these mutagenesis procedures.

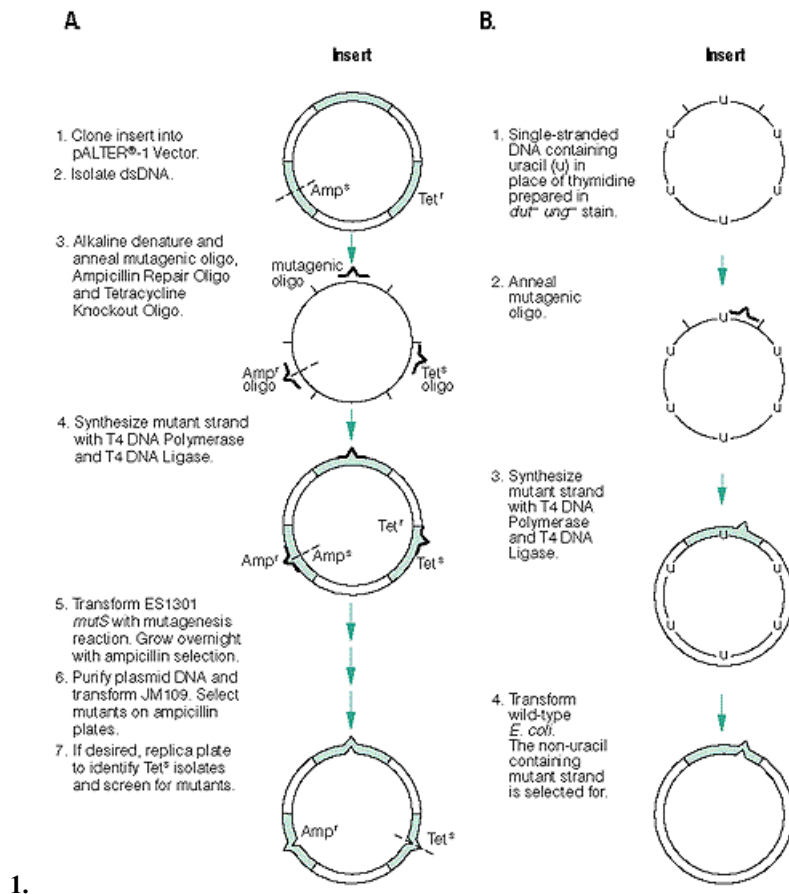


Figure 3. Site-directed mutagenesis using T4 DNA Polymerase. Promega's T4 DNA Polymerase and T4 DNA Ligase were used with standard site-directed mutagenesis techniques. Mutagenic oligonucleotides were hybridized and extended using the T4 DNA Polymerase and the nicks closed by T4 DNA Ligase. **Panel A:** Promega's Altered Sites® II *in vitro* Mutagenesis System, in which the mutant strand is selected by repair of an antibiotic resistance gene. **Panel B:** The system developed by Kunkel (15), in which the parental strand contains uracil residues which are selected against after mutagenesis.

Table 1. Comparison of Site-Directed Mutagenesis Methods that Utilize T4 DNA Polymerase.

Method	Template Source	Mutagenic Oligo	Repair Host	Percent mutant
Altered Sites® II	JM109	+ Mutagenic oligo	JM109	92%
System	JM109	- Mutagenic oligo	JM109	0%

Kunkel	CJ236	+ Mutagenic oligo	JM109	76%
mutagenesis	CJ236	- Mutagenic oligo	JM109	0%

Site-directed mutagenesis using either the Altered Sites® II System or the Kunkel mutagenesis procedure was performed as described (references 16 and 15, respectively).

Summary

Promega's T4 DNA Polymerase is a well characterized enzyme which has many properties that are useful to the molecular biology researcher. The enzyme has very high fidelity, primarily due to the presence of a very active 3'→5' exonuclease activity. This exonuclease activity is also useful for the removal of extended 3' ends and in the creation of 3' recessed termini for incorporation of radiolabeled nucleotides. The enzyme lacks a 5'→3' exonuclease activity and has no strand displacement activity, making it the enzyme of choice for site-directed mutagenesis procedures.

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Ordering Information

Product	Size	Cat. #
T4 DNA Polymerase	100u	M4211
	5000u (5 x 100u)	M4212

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